

Research paper

# Cryo-irradiation as a terminal method for the sterilization of drug aqueous solutions

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## Abstract

The aim of this study is to evaluate the specificities of the irradiation of drugs in frozen aqueous solution. The structures of the degradation products were determined to gain insight into the radiolysis mechanisms occurring in frozen aqueous solutions. Metoclopramide hydrochloride and metoprolol tartrate were chosen as models. The frozen solutions were irradiated at dry ice temperature by high energy electrons at various doses. The drug purity (chemical potency) and the radiolysis products were quantified by HPLC-DAD. Characterization of the degradation products was performed by LC-APCI-MS-MS. The structures of the radiolysis products detected in irradiated frozen aqueous solutions were compared to those detected in solid-state and aqueous solutions (previous studies). For both metoclopramide and metoprolol, solute loss upon irradiation of frozen aqueous solutions was negligible. Five radiolysis products present in traces were identified in irradiated metoclopramide frozen solutions. Three of them were previously identified in solid-state irradiated metoclopramide crystals. The two others were formed following reactions with the hydroxyl radical (indirect effect). Only one fragmentation product was observed in irradiated metoprolol frozen solutions. For both drugs, radiosterilization of frozen solutions, even at high doses (25 kGy), was found to be possible.

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## 1. Introduction

According to the EMEA decision tree for the selection of sterilization methods for liquids, there is no alternative terminal sterilization method to steam irradiation as the alternative method is currently filtration sterilization. Radiosterilization is not used for the sterilization of thermo-sensitive aqueous solutions [1]. An important degradation is observed after irradiation of the drug in solution compared to the solid-state [2] due to the formation of reactive species upon irradiation (mainly  $\cdot\text{OH}$ ,  $\cdot\text{H}$  and  $e_{\text{aq}}^-$  in deaerated water) which react with the solute (indirect effect) [3]. If applicable

to aqueous solutions, radiosterilization would allow sterilization of the drug in its final container. Compared to solid-state irradiation, radiosterilization of aqueous solutions would be more interesting as in the first case, the drug still has to be dissolved prior its injection to patients.

Different approaches may be followed to lower drug degradation. The addition of selected radioprotective excipients partially prevented drug degradation, provided the irradiation dose was decreased [4,5]. The other possibility is to limit the diffusion of the reactive species by a phase transition from the liquid to the solid-state. Irradiation of solutions at subfreezing temperature (cryo-irradiation) might combine the advantages of irradiation at low temperature and solid-state irradiation.

The mechanisms responsible for the formation of radiolysis products in frozen aqueous solutions are not yet fully understood. There is still much controversy on the relative importance of the indirect effect (attack of the solute by the

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reactive species from water) and the direct effect in frozen solutions [6,7]. In order to contribute to the description of the processes underlying the formation of radiolysis products, the structures of the radiolysis products from irradiated frozen aqueous solutions need to be determined and compared to those observed in irradiated solids and aqueous solutions. The aim of this work is to investigate whether cryoirradiation would be feasible from a chemical point of view.

In our previous studies [5,8], the use of ionizing radiation for the sterilization of pharmaceuticals was investigated. In the solid state, metoclopramide has shown to be a suitable candidate for radiosterilization. Radiosterilization of aqueous solutions of metoclopramide aqueous solutions containing radioprotective excipients such as mannitol, vitamin B3 or vitamin B6 was also found to be feasible. However, if radiosterilization of metoclopramide solutions containing these excipients were to be performed, an irradiation dose lower than 25 kGy (ca. 15 kGy) should be validated [9] in order to minimize the loss of chemical potency.

Metoclopramide and metoprolol frozen aqueous solutions were irradiated at various doses (from 11 to 60 kGy) by high energy electrons. The loss of chemical potency as well as the amounts of radiolysis products were quantified by high performance liquid chromatography with a diode array detector (HPLC-DAD). Radiolysis products were characterized by liquid chromatography linked to a tandem mass spectrometer equipped with an atmospheric pressure chemical ionization source (LC-APCI-MS-MS) and are compared to those previously observed after irradiation of metoclopramide crystals and liquid aqueous solutions [5,8]. The radiolysis of frozen metoprolol tartrate solutions was also studied since radiolysis products of this drug in aqueous solution had also been investigated [4,10]. The processes underlying the formation of the radiolysis products in frozen solution are discussed.

## 2. Materials and methods

### 2.1. Materials

Metoclopramide hydrochloride monohydrate (purity >99%) and metoprolol tartrate (purity >99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and stored in the dark at room temperature. Ammonium acetate was from UCB (Brussels, Belgium). HPLC supra-gradient acetonitrile was supplied from Biosolve (Amsterdam, the Netherlands). Deionized water and tri-distilled water were produced in our laboratory. Nitrogen was supplied by Air-Liquide (Liège, Belgium).

### 2.2. Samples for irradiation

Metoclopramide hydrochloride 5 mg ml<sup>-1</sup> (which corresponds to the concentration of the commercial drug) and

Metoprolol tartrate 1 mg ml<sup>-1</sup> solutions were prepared with tri-distilled water, put in glass vials protected from the light, saturated with nitrogen, sealed, frozen and irradiated at dry ice temperature. The temperature of the solution was gradually decreased in order to prevent the glass from breaking.

### 2.3. Irradiation source

Irradiations were performed with a linear electron generator (Mölnlycke, Wareme, Belgium). The dose rate was about  $6 \times 10^7$  Gy h<sup>-1</sup>. The beam power was about 16 kW and the electrons were delivered in pulses of 474 Hz. The desired dose was achieved by adjusting the speed of the conveyor belt. The absorbed doses were 0, 11, 15, 25 and 60 kGy. A polymethylmethacrylate film was pasted on the sample and was used to control the absorbed dose.

The effect of the dose rate on the radiolysis could not be studied as, due to the necessity to maintain a low temperature during the whole irradiation process, irradiation could only be carried out by high energy electrons.

### 2.4. HPLC-DAD system

The Merck-Hitachi (Darmstadt, Germany) HPLC system consisted of a L-6200 Intelligent Pump equipped with an AS-2000 autosampler with a 20 µl sample loop and a L-4500 diode array detector (DAD).

For metoclopramide samples, chromatographic separation was achieved on a 250 × 4 mm Merck LiChrospher® 60 RP Select B column 5 µm particle size. The mobile phase consisted of a mixture of 14% acetonitrile and 86% ammonium acetate 10 mM aqueous solution adjusted to pH 5 with glacial acetic acid at a flow rate of 1 ml min<sup>-1</sup>. The analyses were performed in triplicate. The absorbance was measured between 200 and 700 nm. The wavelength for quantification is 273 nm.

For the analysis of metoprolol solutions, the chromatographic method from Slegers et al. [10] was used. The wavelength for the quantification of metoprolol was 223 nm.

For the quantification of radiolysis products, metoclopramide solutions were diluted twice prior analysis whilst for metoprolol solutions, no dilution was performed. For the assessment of chemical potency, the solutions were diluted 10-fold.

The percentage of drug recovery (chemical potency) was calculated from the ratio of the areas under the curve (AUC) of the drug peak between irradiated and non-irradiated samples [11–13]. For metoclopramide, the concentrations of the degradation products were calculated from a calibration curve and expressed as percentages of the initial drug concentration. Metoclopramide solutions in concentrations ranging from  $8 \times 10^{-7}$  M to  $5 \times 10^{-5}$  M were injected in order to establish the calibration curve which was validated by a statistical analysis (XLStat; Addinsoft, Paris, France). The limits of detection and quantification

were, respectively, 3 and 10 times the signal-to-noise ratio [11,12].

### 2.5. LC–MS–MS system

The LC system consisted in a Waters (Milford, MA, USA) Alliance model 2695 separation module. The chromatographic conditions described in the HPLC DAD part were applied. A Thermofinnigan (Waltham, MA, USA) LCQ Advantage ion trap mass spectrometer was used. The parameters were set as follows: APCI heater temperature, 500 °C; Capillary voltage, 26 V; Capillary temperature, 200 °C; Sheath gas flow, 70 A.U.; Auxiliary gas flow, 30 A.U.; Discharge current 5  $\mu$ A.

### 2.6. pH measurements

A Hanna Instruments (Bedfordshire, UK) pH-meter HI 9025 microcomputer was used. pH was measured at room temperature without any dilution.

## 3. Results

### 3.1. Physico-chemical changes

For both metoclopramide and metoprolol, irradiated samples defrosted at room temperature were colorless. No significant difference in pH was observed between irradiated and unirradiated samples.

### 3.2. Chemical potency

No significant difference was observed between the chemical potency of irradiated frozen solutions and those of unirradiated frozen and non-frozen solutions for both metoclopramide and metoprolol ( $P = 0.75$ ). The drug recovery was  $100 \pm 0.5\%$  for all samples. Therefore, the amounts of degradation products generated following irradiation are expected to be below the error limit.

### 3.3. Degradation products

#### 3.3.1. Metoclopramide

An overlay of the chromatograms of frozen solutions unirradiated and irradiated at 25 kGy is shown in Fig. 1. The radiolysis products were numbered by increasing retention times. Table 1 summarizes the quantification results for the radiolysis products.

**3.3.1.1. Unirradiated solutions.** Two peaks were already present before irradiation of the frozen solution. No significant difference was observed between unirradiated frozen and non-frozen solutions.

**3.3.1.2. Irradiated solutions.** Five degradation peaks were observed after a 25 kGy irradiation. The two peaks present before irradiation increased following irradiation. All

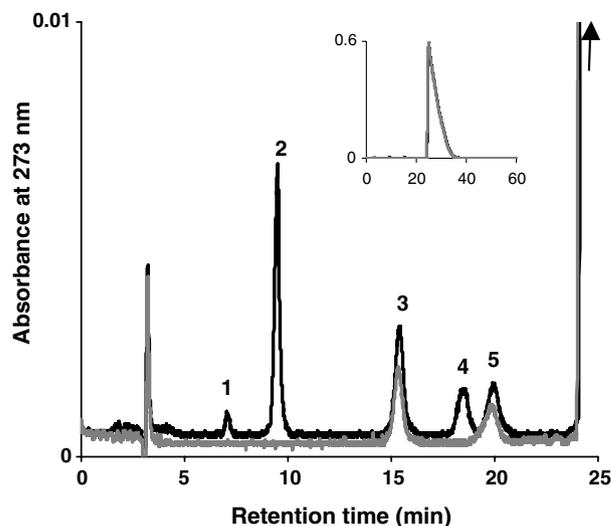


Fig. 1. UV chromatogram zoomed on degradation products for metoclopramide frozen solution unirradiated (lighter) irradiated at 25 kGy by high energy electrons (darker). An extended view is inserted above.

Table 1

Quantification of degradation products as a function of the irradiation dose

	<i>m/z</i>	Mean percentages of peaks ( <i>n</i> = 3)				
		0 kGy	11 kGy	15 kGy	25 kGy	60 kGy
Peak 1	282	<LOD	<LOD	<LOQ	<LOQ	<LOQ
Peak 2	266	<LOD	0.036	0.047	0.086	0.176
Peak 3	272	0.033	0.038	0.044	0.054	0.075
Peak 4	316	<LOD	<LOD	<LOQ	0.024	0.031
Peak 5	286	0.023	0.026	0.027	0.036	0.044

The amounts of degradation products are expressed as percentages of the initial drug concentration.

peaks increased with the irradiation dose. Product 1 was only detectable for irradiation doses above 15 kGy and was still below the quantification limit at 60 kGy.

**3.3.1.3. Characterization of degradation products.** The mass spectrometer indicated the absence of coeluting products in the peaks detected in UV. A product with a *m/z* of 282 was detected in peak 1. Peak 2 consisted in a *m/z* 266 product. Products with *m/z* of 272 and 316 were detected within peaks 3 and 4, respectively, whilst a *m/z* 286 product was observed for peak 5. All the products detected in frozen aqueous solutions of metoclopramide have been previously observed in irradiated metoclopramide liquid solutions [5], which was confirmed by their MS–MS fragmentation patterns. Their structures along with that of metoclopramide are displayed in Fig. 2. Product 1 was formed after the attack of the hydroxyl radical on the chlorine. Product 2 originated from the loss of the chlorine. Products 3 and 5 were obtained after the loss of either the ethyl or the methyl from the lateral chain, respectively. Product 4 was generated after the attack of the hydroxyl radical on the ring. The exact location of the hydroxyl on the ring cannot be

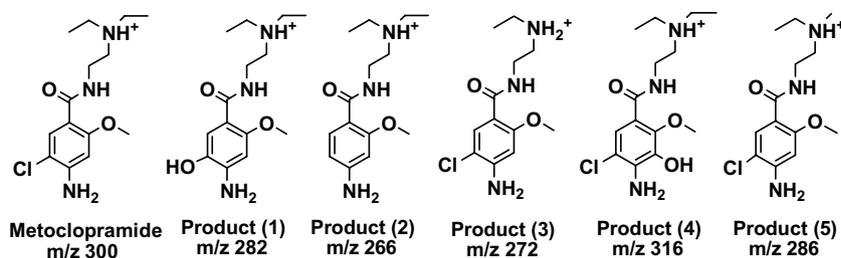


Fig. 2. Structures of metoclopramide and radiolysis products from metoclopramide irradiated frozen solutions (protonated).

determined by mass spectrometry so that the most probable structure according to organic chemistry rules is displayed in Fig. 2.

### 3.3.2. Metoprolol

No degradation peak was detected by HPLC-UV for irradiated frozen solutions of metoprolol tartrate. In order to verify the presence of previously identified degradation products that might be present in so low amounts that they could not be detected by UV, LC-MS was performed on the samples. One product with a  $m/z$  of 226 was detected by the mass spectrometer in irradiated metoprolol frozen solution. The MS-MS fragmentation pattern of this product was similar to that of a product identified in irradiated metoprolol liquid solution [10]. The structure of this product along with that of metoprolol is displayed in Fig. 3.

## 4. Discussion

### 4.1. Chemical potency

The high recoveries observed after irradiation of metoclopramide and metoprolol frozen solutions show the radioresistance of these products when irradiated in frozen aqueous solutions, contrary to the very high degradation that was observed when these drugs were irradiated in non-frozen solution in the absence of any excipient. In liquid solutions, the degradation originated mainly from the reaction of the drug solute with both the hydroxyl radical and the aqueous electron for metoclopramide [5] (ca. 25% loss at 25 kGy), and with the hydroxyl radical for metoprolol [10,14] (ca. 90% loss at 25 kGy).

In frozen aqueous solutions, the diffusion of reactive species from water radiolysis is limited due to the caging effect of the ice lattice [15,16]. In addition, the loss of energy is favored by the regular organization of the mole-

cules and the low temperature favors the reactions with low activation energies such as the recombination of radicals. Therefore, contrary to liquid solutions, the bulk reactive species from ice radiolysis are not able to react readily with the solute.

### 4.2. Yields of degradation products

The irradiation was carried out up to 60 kGy in order to amplify the degradation of the drugs. For metoclopramide, all degradation peaks remain below 0.1% of the initial drug concentration up to a 60 kGy dose at the exception of peak 2 (see Table 1). The radiolysis product detected by LC-MS for metoprolol remained below the limit of UV detection up to a 60 kGy dose. The very low amounts of degradation products detected are in accordance with the fact that no differences were observed between the chemical potency of irradiated and unirradiated samples.

### 4.3. Radiolysis mechanisms

#### 4.3.1. Metoclopramide

**4.3.1.1. Reactions with hydroxyl radicals.** Products 1 and 4 are due to the attack of the hydroxyl radical and were amongst the major radiolysis products formed in irradiated liquid aqueous solutions [5], as they represented about 1.5 and 3.5% of the initial drug concentration, respectively, after irradiation of metoclopramide liquid solution at 15 kGy by gamma rays. In some studies [15,16], radicals due to hydroxylated products have been observed in irradiated frozen aqueous solutions, which shows that reactions with hydroxyl radicals are possible in frozen aqueous solutions. In frozen solutions, the solute molecules might be present as aggregates in ice. Products 1 and 4 might originate from reactions between hydroxyl radicals from ice radiolysis and metoclopramide molecules at the interfaces

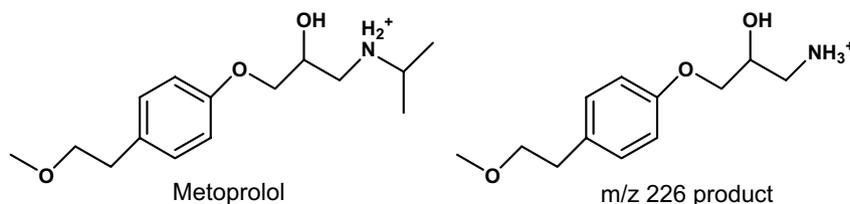


Fig. 3. Structures of metoprolol and radiolysis product.

between ice and aggregated solid metoclopramide. As the diffusion of the hydroxyl radical is very limited or absent in ice [3], it is only able to react with solute molecules from its immediate neighborhood which explains the much lower yields observed for the hydroxylated products. If solute molecules were homogeneously dispersed in the frozen, the yields of these products could be enhanced, so that the low yields are in accordance with the postulate of aggregation. It is therefore conceivable that the other products detected in metoclopramide liquid aqueous solutions might be present at a much lower level in irradiated frozen solutions so that they could not be detected.

**4.3.1.2. Reactions with electrons.** As previously observed [5,8], product 2 is formed after the loss of the chlorine following dissociative electron capture. This product is present in relatively higher amount in irradiated frozen solutions than in irradiated solid-state metoclopramide [8] (0.18% in frozen solutions versus 0.01% in solid metoclopramide after a 25 kGy electron beam irradiation). This might be due to the fact that, in irradiated frozen solutions, trapped electrons from water radiolysis are able to diffuse through a tunneling mechanism so that they may react with the solute molecules.

**4.3.1.3. Fragmentation reactions.** Products 3 and 5 are generated by fragmentation of metoclopramide molecule following the breakage of the ethyl and the methyl from the diethyl amino part. These products are already present before irradiation and were found to increase slightly after irradiation of metoclopramide powder. A similar phenomenon is observed in irradiated frozen solutions.

#### 4.3.2. Metoprolol

In metoprolol irradiated frozen solution, no product due to the attack of the hydroxyl radical was observed. The  $m/z$  226 product has been previously observed in irradiated metoprolol liquid solutions [10]. This product is due to a fragmentation of the lateral chain of metoprolol, which results from the direct effect.

#### 4.3.3. Overview of radiolysis mechanisms

Concerning the mechanisms observed in irradiated frozen solutions, it appears that those involving the attack of the hydroxyl radical are not of significant importance. In irradiated frozen metoclopramide solutions, the most important effect was due to the attack of the electron since metoclopramide contains an electrophilic group (chlorine). Product 2, due to the attack of electron, was present in much greater amounts than both products 1 and 4 which are formed after the attack of the hydroxyl radical. The efficiency of the attack carried out by the electron depends on the physico-chemical properties of the irradiated solid matrix.

Concerning metoprolol, for the degradation of which the attack of the electron does not play a major role, only a fragmentation product was detected in traces. The prod-

ucts due to the attack of the hydroxyl radical, which was the major species responsible for the degradation of metoprolol in irradiated liquid aqueous solutions [10,14], were not detected in irradiated frozen metoprolol solutions. The efficiency of the attack of the hydroxyl radical on the solute in frozen aqueous solution depends on the contact surface between the solute and ice. Further studies concerning the effect of the solute aggregation are needed. Our hypothesis is that contact surfaces are more reduced for metoprolol frozen solutions.

#### 4.4. Feasibility of the cryoirradiation process

From the review of the literature on the radiosensitivity of microorganisms as a function of the irradiation conditions, it appears that microorganisms are less radiosensitive in frozen solutions [17,18]. Therefore, sterilization of frozen solutions by ionizing radiation would probably necessitate a slight increase in the irradiation dose in order to obtain a sufficient sterility assurance level. However, due to the negligible drug degradation observed up to a 60 kGy dose, this is not a major issue to the applicability of this process for the studied compounds. Future studies should be focused on the radiosensitivity of germs found in pharmaceutical media as a function of the temperature so that the guidelines might be adapted to the irradiation conditions.

This method would also necessitate the use of plastic containers which are more suitable than glass for frozen solutions. Although glass is generally considered as a packaging material of choice for parenteral drugs, it darkens on exposure to radiation and is fragile so that it could break during freezing (especially if the process is fast). Therefore, the use of plastics should be preferred over glass for the radiosterilization of frozen solutions. Radioresistant plastics are already available [19,20].

## 5. Conclusion

Cryoirradiation appears to be a suitable method for the sterilization of drugs aqueous solutions and should be considered as a terminal sterilization method, especially for new drugs. Compared to the irradiation of liquid aqueous solutions, even those containing radioprotective excipients, the degradation of the drug is negligible and fewer radiolysis products are detected in traces. This method still needs to be developed and further studies on a wider diversity of compounds will be needed to confirm its potential.

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